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(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: VOLLMOND, Thomas [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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(54) Title: FABRIC TREATED WITH A CELLULASE AND A HYBRID ENZYME COMPRISING A PHENOL OXIDIZING ENZYME

(57) Abstract

The present invention relates to a process of providing a worn but not bleached look in fabrics, comprising a mild treatment with a cellulase and a mild treatment with a hybrid enzyme comprising a phenol oxidizing enzyme.

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# FABRIC TREATED WITH A CELLULASE AND A HYBRID ENZYME COMPRISING A PHENOL OXIDIZING ENZYME

## FIELD OF INVENTION

The present invention relates to a process for providing a worn look in dyed fabric, especially cellulosic fabric such as denim.

## BACKGROUND ART

The past several years have seen the emergence of a new industry, the so called "jeans stonewashing" segment, generated by the fashion demands of a generation desirous of stylish, but informal and comfortable clothing.

Originally, all of the indigo jeans on the market were stiff and uncomfortable when first purchased, due to the finishing system used for denim fabrics.

The first step in the processing evolution was to sell jeans that had been laundered by the manufacturer. These "pre-washed" jeans had a slightly faded appearance and a softer hand that felt comfortable, as though they had been laundered several times. This trend became fashionable as well, and consumers were willing to pay the extra cost involved for this additional processing.

Not long after the introduction of pre-washed jeans, the idea of using abrasive stones to accelerate the aging process was developed, and "stone washing" became the second step in the evolution. Volcanic stones were included in the wash, or tumbled with the damp garments to wear down the stiffest portions such as belt areas, cuffs, and pockets.

However, the use of stones to abrade jeans is very destructive to equipment and fabric, so today the stones are often substituted with a cellulase treatment, or a combination of stones and cellulase is used to achieve the abraded (worn)

look; for reference see "AATCC: Garment Wet Processing Technical Manual", 1994, published by American Association of Textile Chemists and Colorists, pp. 19-21.

The fabric looses strength by using the stone-process described above, and the stone-free cellulase treatment does not alone give the desired worn look, so there is a need in industry for a more gentle process.

**SUMMARY OF THE INVENTION**

Surprisingly it has been found that by combining the cellulase treatment with a treatment with a hybrid enzyme comprising a phenol oxidizing enzyme fused to amino acid sequence having a cellulose-binding domain, it may be possible to achieve the desired worn look in fabric with a minimal strength loss; accordingly, the present invention relates to a process for providing an abraded look with a reduced strength loss in dyed fabric comprising

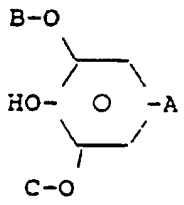
(a) contacting, in an aqueous medium, a dyed fabric with a cellulase in a concentration corresponding to 0.01-250 µg of enzyme protein per g of fabric; and simultaneously or subsequently treating said fabric with a hybrid enzyme comprising a phenol oxidizing enzyme fused to amino acid sequence having a cellulose-binding domain together with a hydrogen peroxide source when the phenol oxidizing enzyme is a peroxidase, or

(b) contacting, in an aqueous medium, a dyed fabric with a hybrid enzyme comprising a phenol oxidizing enzyme fused to amino acid sequence having a cellulose-binding domain together with a hydrogen peroxide source when the phenol oxidizing enzyme is a peroxidase; and subsequently treating said fabric with a cellulase in a concentration corresponding to 0.01-250

µg of enzyme protein per g of fabric.

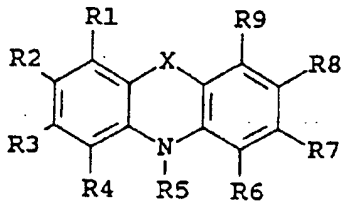
Additionally, it may in some cases be an advantage also to add an enhancing agent to the treatment with the hybrid enzyme mentioned above, said enhancing agent having a formula I or formula II:

Formula I:




in which formula A is a group such as -D, -CH=CH-D, -CH=CH-CH=CH-D, -CH=N-D, -N=N-D, or -N=CH-D, in which D is selected from the group consisting of -CO-E, $-SO_2$-E, -N-XY, and -N$^+$-XYZ, in which E may be -H, -OH, -R, or -OR, and X and Y and Z may be identical or different and selected from -H and -R; R being a $C_1$-$C_{16}$ alkyl, preferably a $C_1$-$C_8$ alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and selected from $C_mH_{2m+1}$; $1 \leq m \leq 5$;

Formula II:




in which formula X represents (-O-) or (-S-), and the substituent groups $R^1$-$R^9$, which may be identical or different, independently represents any of the following radicals:

hydrogen, halogen, hydroxy, formyl, carboxy, and esters and salts hereof, carbamoyl, sulfo, and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, $C_1$-$C_{14}$-alkyl, $C_1$-$C_5$-alkoxy, carbonyl-$C_1$-$C_5$-alkyl, aryl-$C_1$-$C_5$-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group $R^{10}$; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups $R^{10}$; and which $C_1$-$C_{14}$-alkyl, $C_1$-$C_5$-alkoxy, carbonyl-$C_1$-$C_5$-alkyl, and aryl-$C_1$-$C_5$-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups $R^{10}$;

which substituent group $R^{10}$ represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, $C_1$-$C_5$-alkyl, $C_1$-$C_5$-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, $C_1$-$C_5$-alkyl, $C_1$-$C_5$-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which $C_1$-$C_5$-alkyl, and $C_1$-$C_5$-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups $R^1$-$R^9$ may together form a group -B-, in which B represents any of the following the groups: (-$CHR^{10}$-N=N-), (-CH=CH-)$_n$, (-CH=N-)$_n$ or (-N=$CR^{10}$-$NR^{11}$-), in which groups n represents an integer of from 1 to 3, $R^{10}$ is a substituent group as defined above and $R^{11}$ is defined as $R^{10}$.

## DETAILED DESCRIPTION OF THE INVENTION

Bleached versus worn look

Persons skilled in the art of evaluating denim finishing processes, are capable of differentiating between a bleached look and a worn (or abraded) look of denim.

The former is a result of removal (bleaching) of dye from the dyed warp yarn. Since the bleaching takes place on the whole surface of every dyed yarn, the result is a general reduction in colour intensity. Thus, the bleached look of a pair of indigo-dyed jeans is characterised by a lighter blue shade than the corresponding reference.

The latter - the worn look - is a result of a treatment of denim with cellulase and/or pumice stone. This process is characterised by an uneven removal of dye from the fabric, hence it results in a high level of contrast between dyed areas and areas from which dye has been removed.

Typically the worn look is obtained by a process involving cellulase and/or pumice stone, whereas the bleached look can be obtained by a process involving non-enzymatic bleaching agents such as hypochlorite or by a process involving oxidoreductase and an enhancing agent.

The present invention relates to a process of providing a worn but not bleached look, comprising a mild treatment with a cellulase and a subsequent mild treatment with a hybrid enzyme comprising a phenol oxidizing enzyme, or a mild treatment with a hybrid enzyme comprising a phenol oxidizing enzyme followed by a mild treatment with a cellulase, or a simultaneously mild treatment with a cellulase and a hybrid enzyme comprising a phenol oxidizing enzyme.

According to the invention it is preferred to use a cellulase, but instead of using a cellulase pumice stones or perlite may be used, or a combination of cellulase and pumice stones or a combination of cellulase and perlite as known in

the art.

Dyed Fabric

The invention may be applied to any dyed fabric known in the art, in particular to synthetic fabrics such as polyester or to natural fabrics.

The invention is most beneficially applied to cellulose-containing fabrics, such as cotton, viscose, rayon, ramie, linen, Tencel, or mixtures thereof, or mixtures of any of these fibres, or mixtures of any of these fibres together with synthetic fibres. In particular, the fabric is denim.

The fabric may be dyed with vat dyes such as indigo, or indigo-related dyes such as thioindigo. The fabric may also be dyed with more than one dye, e.g., first with a sulphur dye and then with an indigo dye, or vice versa.

In a most preferred embodiment of the invention, the fabric is an indigo-dyed denim with a sulphur-bottom, (i.e. the denim is first dyed with a sulphur dye and then with an indigo dye); including clothing items manufactured therefrom.

Cellulases

In the present context, the term "cellulase" refers to an enzyme which catalyses the degradation of cellulose to glucose, cellobiose, triose and other cello-oligosaccharides.

In the present context the term "cellulase" is understood to include a mature protein or a precursor form thereof or a functional fragment thereof which essentially has the activity of the full-length enzyme. Furthermore, the term "cellulase" is intended to include homologues or analogues of said enzyme. Such homologues comprise an amino acid sequence exhibiting a degree of identity of at least 60% with the amino acid sequence of the parent enzyme, i.e. the parent cellulase. The degree of identity may be determined by conventional methods, see for instance, Altshul et al., Bull. Math. Bio. 48, 1986, pp. 603-616, and Henikoff and Henikoff, Proc. Natl.

Acad. Sci. USA 89, 1992, pp. 10915-10919.

Preferably, the cellulase to be used in the present invention is a monocomponent (recombinant) cellulase, i.e. a cellulase essentially free from other proteins or cellulase proteins. A recombinant cellulase component may be cloned and expressed according to standard techniques conventional to the skilled person.

In a preferred embodiment of the invention, the cellulase to be used in the method is an endoglucanase (EC 3.2.1.4), preferably a monocomponent (recombinant) endoglucanase.

Preferably, the cellulase is a microbial cellulase, more preferably a bacterial or fungal cellulase.

Examples of bacterial cellulases are cellulases derived from or producible by bacteria from the group of genera consisting of Pseudomonas or Bacillus, in particular Bacillus lautus.

The cellulase or endoglucanase may be an acid, a neutral or an alkaline cellulase or endoglucanase, i.e. exhibiting maximum cellulolytic activity in the acid, neutral or alkaline range, respectively.

Accordingly, a useful cellulase is an acid cellulase, preferably a fungal acid cellulase, which is derived from or producible by fungi from the group of genera consisting of Trichoderma, Actinomyces, Myrothecium, Aspergillus, and Botrytis.

A preferred useful acid cellulase is derived from or producible by fungi from the group of species consisting of Trichoderma viride, Trichoderma reesei, Trichoderma longibrachiatum, Myrothecium verrucaria, Aspergillus niger, Aspergillus oryzae, and Botrytis cinerea.

Another useful cellulase or endoglucanase is a neutral or alkaline cellulase, preferably a fungal neutral or alkaline cellulase, which is derived from or producible by fungi from the group of genera consisting of Aspergillus,

Penicillium, Myceliophthora, Humicola, Irpex, Fusarium, Stachybotrys, Scopulariopsis, Chaetomium, Mycogone, Verticillium, Myrothecium, Papulospora, Gliocladium, Cephalosporium and Acremonium.

A preferred alkaline cellulase is derived from or producible by fungi from the group of species consisting of Humicola insolens, Fusarium oxysporum, Myceliopthora thermophila, or Cephalosporium sp., preferably from the group of species consisting of Humicola insolens, DSM 1800, Fusarium oxysporum, DSM 2672, Myceliopthora thermophila, CBS 117.65, or Cephalosporium sp., RYM-202.

A preferred example of a native or parent cellulase is an alkaline endoglucanase which is immunologically reactive with an antibody raised against a highly purified ≅43kD endoglucanase derived from Humicola insolens, DSM 1800, or which is a derivative of the ≅43kD endoglucanase exhibiting cellulase activity.

Other examples of useful cellulases are variants having, as a parent cellulase, a cellulase of fungal origin, e.g. a cellulase derivable from a strain of the fungal genus Humicola, Trichoderma or Fusarium.

According to the invention the concentration of the cellulase enzyme in the aqueous medium may be 0.01-250 µg of enzyme protein per g of fabric, in particular 0.1-50 µg of enzyme protein per g of fabric.

Determination of cellulase activity (ECU)

In the context of this invention, cellulase activity can be expressed in ECU. Cellulolytic enzymes hydrolyse CMC, thereby increasing the viscosity of the incubation mixture. The resulting reduction in viscosity may be determined by a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France).

Determination of the cellulolytic activity, measured in terms of ECU, may be determined according to the following

analysis method (assay): The ECU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C; pH 7.5; 0.1M phosphate buffer; time 30 min; using a relative enzyme standard for reducing the viscosity of the CMC (carboxymethylcellulose Hercules 7 LFD) substrate; enzyme concentration approx. 0.15 ECU/ml. The arch standard is defined to 8200 ECU/g.

Enzyme hybrids

According to the invention an enzyme hybrid comprises a phenol oxidizing enzyme fused to an amino acid sequence having a cellulose-binding domain.

By the term "a phenol oxidizing enzyme" is meant an enzyme, which by using hydrogen peroxide or molecular oxygen, is capable of oxidizing organic compounds containing phenolic groups. Examples of such enzymes are peroxidases and oxidases.

Enzyme hybrids are known in the art, (for reference see e.g. WO 90/00609 and WO 95/16782): They may be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding the enzyme of interest and growing the host cell to express the fused gene. The enzyme hybrids may be described by the following formula:

CBD - MR - X,

wherein:

CBD can be either the N-terminal or the C-terminal region of an amino acid sequence corresponding to at least the cellulose-binding domain;

MR is the middle region (the linker), and may be a bond, or a short linking group of from about 2 to about 100 carbon atoms, in particular of from 2 to 40 carbon atoms, or

typically from about 2 to about 100 amino acids, in particular of from 2 to 40 amino acids, and

X can be either the N-terminal or the C-terminal region and is a phenol oxidizing enzyme.

Cellulose-Binding Domains

The term "cellulose-binding domain" is intended to be understood as defined by Peter Tomme et al. "Cellulose-Binding Domains: Classification and Properties" in "Enzymatic Degradation of Insoluble Carbohydrates", John N. Saddler and Michael H. Penner (Eds.), ACS Symposium Series, No. 618, 1996.

This definition classifies more than 120 cellulose-binding domains (CBDs) into 10 families (I-X), and it demonstrates that CBDs are found in various enzymes such as cellulases, xylanases, mannanases, arabinofuranosidases, acetyl esterases and chitinases. CBDs have also been found in algae, e.g., the red alga Porphyra purpurea as a non-hydrolytic polysaccharide-binding protein, for reference see Peter Tomme et al., supra.

However, most of the CBDs are from cellulases and xylanases. CBDs are found at the N or C termini of proteins or are internal.

Cellulases useful for preparation of Cellulose-Binding Domains

The techniques used in isolating a cellulase gene are well known in the art.

In the present context, the term "cellulase" refers to an enzyme which catalyses the degradation of cellulose to glucose, cellobiose, triose and other cello-oligosaccharides.

In a preferred embodiment of the invention, the cellulase to be used in the method is an endoglucanase (EC 3.2.1.4), preferably a monocomponent (recombinant) endoglucanase.

Preferably, the cellulase is a microbial cellulase, more preferably a bacterial or fungal cellulase.

Useful examples of bacterial cellulases are cellulases derived from or producible by bacteria from the group consisting of Pseudomonas, Bacillus, Cellulomonas, Clostridium, Microspora, Thermotoga, Caldocellum and Actinomycets such as Streptomyces, Termomonospora and Acidothemus, in particular from the group consisting of Pseudomonas cellulolyticus, Bacillus lautus, Cellulomonas fimi, Microspora bispora, Termomonospora fusca, Termomonospora cellulolyticum and Acidothemus cellulolyticus.

The cellulase may be an acid, a neutral or an alkaline cellulase, i.e. exhibiting maximum cellulolytic activity in the acid, neutral or alkaline range, respectively.

A useful cellulase is an acid cellulase, preferably a fungal acid cellulase, which is derived from or producible by fungi from the group of genera consisting of Trichoderma, Myrothecium, Aspergillus, Phanaerochaete, Neurospora, Neocallimastix and Botrytis.

A preferred useful acid cellulase is derived from or producible by fungi from the group of species consisting of Trichoderma viride, Trichoderma reesei, Trichoderma longibrachiatum, Myrothecium verrucaria, Aspergillus niger, Aspergillus oryzae, Phanaerochaete chrysosporium, Neurospora crassa, Neocallimastix partriciarum and Botrytis cinerea.

Another useful cellulase is a neutral or alkaline cellulase, preferably a fungal neutral or fungal alkaline cellulase, which is derived from or producible by fungi from the group of genera consisting of Aspergillus, Penicillium, Myceliophthora, Humicola, Irpex, Fusarium, Stachybotrys, Scopulariopsis, Chaetomium, Mycogone, Verticillium, Myrothecium, Papulospora, Gliocladium, Cephalosporium and Acremonium.

A preferred alkaline cellulase is derived from or producible by fungi from the group of species consisting of Humicola insolens, Fusarium oxysporum, Myceliopthora thermophila, Penicillium janthinellum and Cephalosporium sp., preferably from the group of species consisting of Humicola

insolens, DSM 1800, Fusarium oxysporum, DSM 2672, Myceliophthora thermophila, CBS 117.65, and Cephalosporium sp., RYM-202.

A preferred example of a native or parent cellulase is an alkaline endoglucanase which is immunologically reactive with an antibody raised against a highly purified ~43kD endoglucanase derived from Humicola insolens, DSM 1800, or which is a derivative of the ~43kD endoglucanase exhibiting cellulase activity.

Other examples of useful cellulases are variants having, as a parent cellulase, a cellulase of fungal or bacterial origin, e.g. a cellulase derivable from a strain of the fungal genus Humicola, Trichoderma or Fusarium.

The techniques used in isolating a xylanase gene, a mannanase gene, an arabinofuranosidase gene, an acetyl esterase gene or a chitinase gene are also well known in the art.

Isolation of a Cellulose-Binding domain

In order to isolate the cellulose binding domain of e.g. a cellulase, several genetic approaches may be used. One method uses restriction enzymes to remove a portion of the gene and then to fuse the remaining gene-vector fragment in frame to obtain a mutated gene that encodes a protein truncated for a particular gene fragment. Another method involves the use of exonucleases such as Ba131 to systematically delete nucleotides either externally from the 5' and the 3' ends of the DNA or internally from a restricted gap within the gene. These gene deletion methods result in a mutated gene encoding a shortened gene molecule which may then be evaluated for substrate binding ability. Appropriate substrates for evaluating the binding activity include compounds such as Avicel and cotton fibres.

Once a nucleotide sequence encoding the substrate binding region has been identified, either as cDNA or chromosomal DNA, it may then be manipulated in a variety of

ways to fuse it to a DNA sequence encoding the enzyme of interest. The cellulose binding encoding fragment and the DNA encoding the enzyme of interest are then ligated with or without a linker. The resulting ligated DNA may then be manipulated in a variety of ways to provide for expression. Microbial hosts such as Aspergillus, e.g., A. niger and A. oryzae, Bacillus, E. coli or S.cerevisiae are preferred.

Peroxidases

Suitable peroxidases to be fused with the sequence encoding the cellulose-binding domain may be any peroxidase enzyme comprised by the enzyme classification (EC 1.11.1.7), or any fragment derived therefrom, exhibiting peroxidase activity.

Preferably, the peroxidase employed in the method of the invention is producible by plants (e.g. horseradish or soybean peroxidase) or microorganisms such as fungi or bacteria. Some preferred fungi include strains belonging to the subdivision Deuteromycotina, class Hyphomycetes, e.g., Fusarium, Humicola, Tricoderma, Myrothecium, Verticillum, Arthromyces, Caldariomyces, Ulocladium, Embellisia, Cladosporium or Dreschlera, in particular Fusarium oxysporum (DSM 2672), Humicola insolens, Trichoderma resii, Myrothecium verrucana (IFO 6113), Verticillum alboatrum, Verticillum dahlie, Arthromyces ramosus (FERM P-7754), Caldariomyces fumago, Ulocladium chartarum, Embellisia alli or Dreschlera halodes.

Other preferred fungi include strains belonging to the subdivision Basidiomycotina, class Basidiomycetes, e.g. Coprinus, Phanerochaete, Coriolus or Trametes, in particular Coprinus cinereus f. microsporus (IFO 8371), Coprinus macrorhizus, Phanerochaete chrysosporium (e.g. NA-12) or Trametes (previously called Polyporus), e.g. T. versicolor (e.g. PR4 28-A).

Further preferred fungi include strains belonging to the subdivision Zygomycotina, class Mycoraceae, e.g. Rhizopus or Mucor, in particular Mucor hiemalis.

Some preferred bacteria include strains of the order Actinomycetales, e.g., Streptomyces spheroides (ATTC 23965), Streptomyces thermoviolaceus (IFO 12382) or Streptoverticillum verticillium ssp. verticillium.

Other preferred bacteria include Bacillus pumilus (ATCC 12905), Bacillus stearothermophilus, Rhodobacter sphaeroides, Rhodomonas palustri, Streptococcus lactis, Pseudomonas purrocinia (ATCC 15958) or Pseudomonas fluorescens (NRRL B-11).

Further preferred bacteria include strains belonging to Myxococcus, e.g., M. virescens.

Particularly, a recombinantly produced peroxidase is preferred, e.g., a peroxidase derived from a Coprinus sp., in particular C. macrorhizus or C. cinereus according to WO 92/16634, or a variant thereof, e.g., a variant as described in WO 94/12621.

Laccases and Laccase Related Enzymes

Suitable laccases to be fused with the sequence encoding the cellulose-binding domain include laccases and laccase related enzymes, i.e., any laccase enzyme comprised by the enzyme classification (EC 1.10.3.2), any chatechol oxidase enzyme comprised by the enzyme classification (EC 1.10.3.1), any bilirubin oxidase enzyme comprised by the enzyme classification (EC 1.3.3.5) or any monophenol monooxygenase enzyme comprised by the enzyme classification (EC 1.14.18.1).

The laccase enzymes are known from microbial and plant origin. The microbial laccase enzyme may be derived from bacteria or fungi (including filamentous fungi and yeasts) and suitable examples include a laccase derivable from a strain of Aspergillus, Neurospora, e.g., N. crassa, Podospora, Botrytis, Collybia, Fomes, Lentinus, Pleurotus, Trametes, e.g., T. vil-

losa and T. versicolor, Rhizoctonia, e.g., R. solani, Coprinus, e.g. C. plicatilis and C. cinereus, Psatyrella, Myceliophthora, e.g. M. thermophila, Scytalidium, Polyporus, e.g., P. pinsitus, Phlebia, e.g., P. radita (WO 92/01046), or Coriolus, e.g., C. hirsutus (JP 2-238885), in particular laccases obtainable from Trametes, Myceliophthora, Scytalidium or Polyporus.

Plasmids

Preparation of plasmids capable of expressing fusion proteins having the amino acid sequences derived from fragments of more than one polypeptide are well known in the art, for reference see e.g. WO 90/00609. The expression cassette may be included within a replication system for episomal maintenance in an appropiate cellular host or may be provided without a replication system, where it may become integrated into the host genome. The DNA may be introduced into the host in accordance with known techniques such as transformation, microinjection or the like.

Once the fused gene has been introduced into the appropiate host, the host may be grown to express the fused gene. Normally it is desirable additionally to add a signal sequence which provides for secretion of the fused gene.

Typical examples of useful fused genes are:

Signal sequence -- (pro-peptide) -- Cellulose-Binding Domain -- Linker -- Enzyme of interest, or

Signal sequence -- (pro-peptide) -- Enzyme of interest -- Linker -- Cellulose-Binding Domain, in which the pro-peptide normally contains 5-25 amino acids.

The recombinant product may be glycosylated or non-glycosylated.

Phenol Oxidizing Enzyme Systems

If the phenol oxidizing enzyme requires a source of hydrogen peroxide, the source may be hydrogen peroxide or a hydrogen peroxide precursor for in situ production of hydrogen peroxide, e.g., percarbonate or perborate, or a hydrogen peroxide generating enzyme system, e.g. an oxidase and a substrate for the oxidase, or an amino acid oxidase and a suitable amino acid, or a peroxycarboxylic acid or a salt thereof. Hydrogen peroxide may be added at the beginning of or during the process, e.g. in a concentration corresponding to 0.001-25 mM $H_2O_2$.

If the phenol oxidizing enzyme requires molecular oxygen, molecular oxygen from the atmosphere will usually be present in sufficient quantity.

According to the invention the concentration of the hybrid enzyme in the aqueous medium where the localized variation in the colour density of the surface of the dyed fabric is taking place, may be 0.01-250 µg of hybrid enzyme protein per g of fabric, preferably 0.1-50 µg of hybrid enzyme protein per g of fabric.

If the phenol oxidizing enzyme is a peroxidase, the peroxidase activity may be determined in the following way:

1 peroxidase unit (PODU) is the amount of enzyme that catalyzes the conversion of 1 µmole hydrogen peroxide per minute at the following analytical conditions: 0.88 mM hydrogen peroxide, 1.67 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate), 0.1 M phosphate buffer, pH 7.0, incubated at 30°C, photometrically followed at 418 nm.

If the phenol oxidizing enzyme is a laccase, the laccase activity may be determined in the following way:

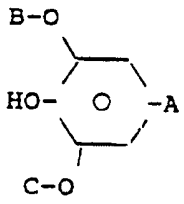
Laccase activity is determined from the oxidation of syringaldazin under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions

are 19 μM syringaldazin, 23.2 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time. 1 laccase unit (LACU) is the amount of enzyme that catalyses the conversion of 1.0 μmole syringaldazin per minute at these conditions.

Enhancing Agents

According to the present invention an enhancing agent is any compound that enhances the bleaching process. The enhancing agent will typically be an organic compound, e.g., an organic compound described by one of the following formulas:

The enhancing agent may be described by the following formula I:




in which formula A is a group such as -D, -CH=CH-D, -CH=CH-CH=CH-D, -CH=N-D, -N=N-D, or -N=CH-D, in which D is selected from the group consisting of -CO-E, $-SO_2$-E, -N-XY, and -N$^+$-XYZ, in which E may be -H, -OH, -R, or -OR, and X and Y and Z may be identical or different and selected from -H and -R; R being a $C_1$-$C_{16}$ alkyl, preferably a $C_1$-$C_8$ alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and selected from $C_mH_{2m+1}$; $1 \leq m \leq 5$.

In a preferred embodiment A in the above mentioned formula is -CO-E, in which E may be -H, -OH, -R, or -OR; R being a $C_1$-$C_{16}$ alkyl, preferably a $C_1$-$C_8$ alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and

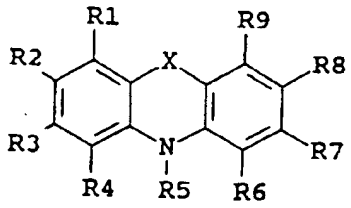
optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and selected from $C_mH_{2m+1}$; $1 \leq m \leq 5$.

In the above mentioned formula A may be placed meta to the hydroxy group instead of being placed in the para-position as shown.

In particular embodiments, the enhancing agent is acetosyringone, methylsyringate, ethylsyringate, propyl-syringate, butylsyringate, hexylsyringate, or octylsyringate.

The enhancing agents described above may be prepared using methods well known to those skilled in the art; some of the enhancing agents are also commercially available, e.g., acetosyringone. Methylsyringate, ethylsyringate, propyl-syringate, butylsyringate, hexylsyringate and octylsyringate may be produced as disclosed in Chem. Ber. 67, 1934, p. 67.

The enhancing agent used in the present invention may also be described by the following formula II:




in which formula X represents (-O-) or (-S-), and the substituent groups $R^1$-$R^9$, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy, and esters and salts hereof, carbamoyl, sulfo, and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, $C_1$-$C_{14}$-alkyl, $C_1$-$C_5$-alkoxy, carbonyl-$C_1$-$C_5$-alkyl, aryl-$C_1$-$C_5$-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group $R^{10}$; and which phenyl may furthermore be unsubstituted or

substituted with one or more substituent groups $R^{10}$; and which $C_1$-$C_{14}$-alkyl, $C_1$-$C_5$-alkoxy, carbonyl-$C_1$-$C_5$-alkyl, and aryl-$C_1$-$C_5$-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups $R^{10}$;

which substituent group $R^{10}$ represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, $C_1$-$C_5$-alkyl, $C_1$-$C_5$-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, $C_1$-$C_5$-alkyl, $C_1$-$C_5$-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which $C_1$-$C_5$-alkyl, and $C_1$-$C_5$-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups $R^1$-$R^9$ may together form a group -B-, in which B represents any of the following the groups: (-$CHR^{10}$-N=N-), (-CH=CH-)$_n$, (-CH=N-)$_n$ or (-N=$CR^{10}$-$NR^{11}$-), in which groups n represents an integer of from 1 to 3, $R^{10}$ is a substituent group as defined above and $R^{11}$ is defined as $R^{10}$.

In particular embodiments, the enhancing agent is 10-methylphenothiazine, phenothiazine-10-propionic acid, N-hydroxysuccinimide phenothiazine-10-propionate, 10-ethylphenothiazine-4-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl phenothiazine-10-propionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methylpiperazin-1-yl)propyl)phenothiazine,

10-(2-pyrrolidin-1-yl-ethyl)phenothiazine, 2-methoxy-10-methyl-phenothiazine, 1-methoxy-10-methylphenothiazine, 3-methoxy-10-methylphenothiazine, 3,10-dimethylphenothiazine, 3,7,10-trimethylphenothiazine, 10-(2-hydroxyethyl)phenothiazine, 10-(3-hydroxypropyl)phenothiazine, 3-(2-hydroxyethyl)-10-methylphenothiazine, 3-hydroxymethyl-10-methylphenothiazine, 3,7-dibromophenothiazine-10-propionic acid, phenothiazine-10-propionamide, chlorpromazine, 2-chloro-10-methylphenothiazine, 2-acetyl-10-methylphenothiazine, 10-methylphenoxazine, 10-ethylphenoxazine, phenoxazine-10-propionic acid, 10-(2-hydroxyethyl)phenoxazine or 4-carboxyphenoxazine-10-propionic acid.

The enhancing agents may be obtained from Sigma-Aldrich, Janssen Chimica, Kodak, Tokyo Kasai Organic Chemicals, Daiichi Pure Chemicals Co. or Boehringer Mannheim; N-methylated derivatives of phenothiazine and phenoxazine may be prepared by methylation with methyliodide as described by Cornel Bodea and Ioan Silberg in "Recent Advances in the Chemistry of Phenothiazines" (Advances in heterocyclic chemistry, 1968, Vol. 9, pp. 321-460); B. Cardillo & G. Casnati in Tetrahedron, 1967, Vol. 23, p. 3771. Phenothiazine and phenoxazine propionic acids may be prepared as described in J. Org. Chem. 15, 1950, pp. 1125-1130. Hydroxyethyl and hydroxypropyl derivatives of phenothiazine and phenoxazine may be prepared as described by G. Cauquil in Bulletin de la Society Chemique de France, 1960, p.1049.

The enhancing agent of the invention may be present in concentrations of from 0.05 to 500 µmole per g of fabric, preferably 0.05 to 100 µmole per g of fabric, more preferably 0.05 to 10 µmole per g of fabric.

Industrial Applications

The present invention is typically used in industrial machines for cellulase treatment of fabric.

The fabric is normally added to the machine according to the machine capacity per the manufacturer's instructions. The fabric may be added to the machine prior to introducing water or the fabric may be added after water is introduced.

Normally, the cellulase treatment will be performed first, followed by the treatment with the enzyme hybrid and optionally the enhancing agent, but the two processes may be performed simultaneously, or vice versa.

The cellulase may be present in the water prior to adding the fabric or it may be added after the fabric has been wetted. Normally a buffer will be used in order to be close to the pH optimum of the enzyme in question. After the fabric has been contacted with the cellulase it should be agitated in the machine for a sufficient period of time to ensure that the fabric is fully wetted and to ensure the action of the enzyme. Typically a reaction time between 5 and 60 minutes and a reaction temperature between 20°C and 90°C, preferably between 30°C and 80°C, more preferably between 40°C and 70°C, will be suitable.

The enzyme hybrid, the hydrogen peroxide source if the enzyme hybrid comprises a peroxidase, and optionally the enhancing agent of the invention may be present in the water prior to adding the fabric or they may be added after the fabric has been wetted. The enzyme hybrid may be added simultaneously with the enhancing agent or they may be added separately. Normally a buffer will be used in order to be close to the pH optimum of the enzyme in question. After the fabric has been contacted with the hybrid enzyme, if necessary a hydrogen peroxide source, and optionally the enhancing agent of the invention it should be agitated in the machine for a sufficient period of time to ensure that the fabric is fully wetted and to ensure the action of the enzyme and the enhancing agent. Typically a reaction time between 5 and 60

minutes and a reaction temperature between 20°C and 90°C, preferably between 30°C and 80°C, more preferably between 40°C and 70°C, will be suitable.

The above described process steps may be performed once or it may be repeated two or three times depending on how worn the dyed fabric should look.

Visual Test Procedure

A person skilled in the art knows whether, e.g., a pair of jeans looks bleached or worn, so when testing the new process according to the invention it is best to let skilled persons do a visual evaluation comparing fabric treated with one of the traditional methods with fabric treated according to the present invention.

Technical Test Procedure

In addition to the visual inspection described above the evaluation of the worn look may be quantified by using a Minolta Chroma Meter CR200 or a Minolta Chroma Meter CR300.

Evaluation: A Minolta Chroma Meter CR200 or CR300 (available from Minolta Corp.) is used according to Manufacturer's instructions to evaluate the degree of abrasion as well as to estimate any discoloration using the change in the colour space coordinates $L^*a^*b^*$ (CIELAB-system): $L^*$ gives the change in white/black at a scale of from 0 to 100, $a^*$ gives the change in green ($-a^*$)/red ($+a^*$), and $b^*$ gives the change in blue ($-b^*$)/yellow ($+b^*$). A decrease in $L^*$ means an increase in black colour (decrease of white colour), an increase in $L^*$ means an increase in white colour (a decrease in black colour), a decrease in $a^*$ means an increase in green colour (decrease in red colour), an increase in $a^*$ means an increase in red colour (a decrease in green colour), a decrease in $b^*$ means an increase in blue colour (a decrease in yellow colour), and an

increase in b* means an increase in yellow colour (a decrease in blue colour).

The fabric swatches treated according to the invention are compared to non-treated fabric swatches.

The Minolta Chroma Meter CR200 or the Minolta Chroma Meter CR300 is operated in the L*a*b* colour space (coordinate system). The light source used is a CIE light standard C. Each measurement is an average of 3 measurements. The instrument is calibrated using a Minolta calibration plate (white). 10 non-treated denim swatches are measured 2 times each and the average of the coordinates L*a*b* are calculated and entered as a reference. The coordinates of the samples are then calculated as the difference (Δ) of the average of 3 measurements on each swatch from the reference value of the coordinates L*a*b*.

The invention is further illustrated in the following examples which are not in any way intended to limit the scope of the invention as claimed.

**EXAMPLE 1**

Construction of a peroxidase fused to a cellulose binding domain (CiP-CBD and mCiP842-CBD)

This example concerns fusion proteins comprising a CBD linked to *Coprinus cinereus* peroxidase (CiP) or to a mutant thereof (mCiP842) (see, e.g., WO 95/10602).

**Yeast expression system**

The pJC106/YNG344 host/vector system was chosen as the standard expression system for all CiP experiments utilizing yeast expression. Mutant mCiP842 contains the following amino acid substitutions relative to the parent CiP: V53A, E239G, M242I, Y272F.

**Construction of the CBD-CiP fusion vector JC20A or JC20D: CiP signal seq.-*H. insolens* family 45 cellulase CBD-*H. insolens* family 45 cellulase linker-CiP, or CiP signal seq.-*H. insolens* family 45 cellulase CBD-*H. insolens* family 45 cellulase linker-mCiP842.**

The CBD-CiP fusion was constructed by amplifying four separate gene fragments using PCR. A) The CiP 5'-untranslated region and the CiP coding sequence from plasmid JC106 or mCiP842 encoding amino acids 1 to 22, B) the *H. insolens* family 45 cellulase CBD from plasmid pCaHj418 encoding amino acids 248-305, C) the *H. insolens* family 45 cellulase linker domain from plasmid pCaHj418 encoding amino acids 213-247, and D) the CiP coding sequence from plasmid JC106 or mCiP842 encoding amino acids 21 to 344.

The sequence of the *H. insolens* family 45 cellulase is disclosed in WO 91/17244.

Primers used in amplifications A through D were as follows:

Amplification A:

1. CiPpcrdwn: CCCCCTTCCCTGGCGAATTCCGCATGAGG
2. JC20.1: ACCTTGGGGTAGAGCGAGGGCACCGATG

Amplification B:

3. JC20.2: TGCACTGCTGAGAGGTGGGC
4. JC20.3: CAGGCACTGATGATACCAGT

Amplification C:

5. JC20.4: CCCTCCAGCAGCACCAGCTCT
6. JC20.5: TCCTCCAGGACCCTGACCGCTCGGAGTCGTAGGCTG

Amplification D:

7. JC20.6: TACGACTCCGAGCGGTCAGGGTCCTGGAGGAGGCGGG
8. YES2term: GGGAGGGCGTGAATGTAAG

Amplified products of reactions A) and B) were purified

and phosphorylated using T4 polynucleotide kinase, ligated to one another for 15 min. at room temperature, and amplified with primers 1 and 4 to generate product AB. Amplified products of reactions C) and D) were purified and mixed, then PCR-amplified to generate product CD. Reaction products AB and CD were purified and phosphorylated using T4 polynucleotide kinase, ligated to one another for 15 min. at room temperature, and amplified with primers 1 and 8 to generate the final product. The resulting product was purified, mixed with plasmid JC106 which had the CiP gene removed by digestion with BamHI and XhoI. Plasmid JC20A contains the wild type CiP gene, whereas plasmid JC20D contains the peroxide-stable mutant mCiP842. Transformants were selected on minimal media lacking uridine.

**Scoring of transformants for peroxidase and cellulose-binding activity**

*Plate Assay:* Yeast transformants were grown on minimal media plates containing 2% galactose (to induce the GAL1 yeast promoter driving CBD-CiP expression) that had been covered with a double filter layer consisting of cellulose acetate on top of nitrocellulose. After overnight growth, both filters were washed twice with 100 ml of 20 mM phosphate buffer, pH 7.0 for 5 minutes, after which no colony debris could be detected. Filters were then assayed for bound peroxidase activity by coating them with a 100 mM phosphate buffer, pH 7.0, containing 50 µg/ml of diamino-benzidine and 1 mM hydrogen peroxide. Bound peroxidase activity appears as a brown precipitate on the filter.

*Liquid Assay:* Liquid cultures of mutants demonstrating cellulose binding in the filter assay were grown overnight in minimal media containing 2% galactose. 20 µl samples of culture broth were mixed with Avicel crystalline cellulose (20 g/L) in 0.1 M phosphate buffer, pH 7, 0.01% Tween 20 in

a total volume of 100 µl and incubated at 22°C for 10 minutes. The mixture was then centrifuged to pellet the insoluble cellulose fraction, and the supernatants were assayed for peroxidase activity. Binding was scored as the % activity bound to the insoluble cellulose fraction based on the decrease in soluble activity.

**High pH/thermal stability screening of CBD-CiP fusions**

This screening process utilizes broth samples from yeast cultures grown in microtiter plates. The 96-well plate screen is performed by first growing yeast transformants of a pool of mutants in 50 µL volumes of URA(-) medium, pH 6.0 in 96-well microtiter plates. Cultures are inoculated by dilution into medium and pipetting (robotic or manual autopipettor) into 96-well plates. These are placed in an incubator set at 30°C, 350 RPM and shaken for approximately 5 days. Plates are placed directly from the culture box onto the robotic system.

Both CiP and mCiP842 and the related fusion proteins were subjected to a combined pH - temperature - $H_2O_2$ stress test: After an initial activity assay, cultures are diluted to ca. 0.06 PODU/ml, and incubated in 200 µM hydrogen peroxide, 100 mM phosphate/borate buffer, pH 10.5 at 50°C. After 0, 10, 20 and 30 minutes, samples are removed and residual activity is measured using the standard ABTS assay, pH 7.0. Improved mutants are those showing higher residual activity than CiP and are expressed as percent residual activity relative to the time 0 assay result.

Yeast expression plasmids designed to make *H. insolens* family 45 cellulase CBD-CiP fusions were constructed and sequenced.

These JC20-series plasmids were transformed into *S. cerevisae* for expression and testing. After transformation, yeast colonies were grown on selective plates covered with a

double filter layer: cellulose acetate filters on top of nitrocellulose. Wild type CiP secreted from yeast JC106 and the stable mutant mCiP842 pass through the cellulose acetate, then binds to the nitrocellulose where it can be visualized using diaminobenzidine (DAB) and $H_2O_2$. The cellulose acetate filter does not bind any wild-type or mCiP842 peroxidase. In contrast, the N-terminal CBD-CiP fusions encoded by plasmids JC20A, and JC20D are all detectable on both filters using the DAB assay, indicating that the fusion proteins have both peroxidase and cellulose-binding activities. In all cases the peroxidase activity bound to the cellulose acetate filter remains bound even after washing extensively with buffer at pH 7. The activity bound to the lower nitrocellulose filter suggests that binding of the CBD-CiP may be incomplete, or the cellulose filter gets saturated, allowing some of the fusion protein to pass through to the lower filter, or that some percentage of the fusion protein gets truncated to include only the peroxidase domain.

**EXAMPLE 2**

Abrasion enhancement by treating with cellulase and CBD-oxidoreductase (CiP-CBD)

San Francisco denim fabric (standard sulphur bottom denim fabric from Swift, France) was desized with Aquazym (available from Novo Nordisk A/S) and abraded with DeniMax T (available from Novo Nordisk A/S) to obtain a mildly abraded look. The resulting L*a*b* values of the San Francisco denim after abrasion was: L*: 30.87; a*: -0.16; b*: -15.36

Dakota denim fabric (standard pure indigo denim fabric from Swift, France) was desized with Aquazym (available from Novo Nordisk A/S) and abraded with DeniMax T (available from Novo Nordisk A/S) to obtain a mildly abraded look. The resulting L*a*b* values of the Dakota denim after abrasion was: L*: 28.95; a*: 0.76; b*: -17.90.

**Abrasion enhancement using CiP-CBD:**

The following was performed in a Wascator FOM 71 MP-LAB:
660 g denim (approximately 495 g of the above mildly abraded San Francisco denim, and approximately 165 g of the above mildly abraded Dakota denim),
16.5 litres of de-mineralised water,
12.38 g $KH_2PO_4$,
CiP-CBD (produced according to Example 1): 3 µg enzyme protein per g denim,
0.0825 g methyl syringate (Lancaster) corresponding to 0.60 µmole/g denim,
1.685 ml 30% $H_2O_2$ (Perhydrol, Merck) corresponding to 1 mM $H_2O_2$,
pH 6.0,
50°C,
30 minutes.

The resulting L*a*b* values of the denim was:
San Francisco denim: L*: 33.65; a*: -0.30; b*: -15.07.
Dakota denim: L*: 31.85; a*: 0.25; b*: -17.06.

The change in L*a*b* was:
San Francisco denim: ΔL*: 2.78; Δa*: -0.14; Δb*: 0.29.
Dakota denim: L*: 2.90; a*: -0.51; b*: 0.84.

From visual evaluation the abrasion enhancement process produced denim with a more worn (more abraded) look rather than a bleached look.

**EXAMPLE 3**

Abrasion enhancement by treating with cellulase and CBD-oxidoreductase (mCiP842-CBD)

San Francisco denim fabric (standard sulphur bottom denim

fabric from Swift, France) was desized with Aquazym (available from Novo Nordisk A/S) and abraded with DeniMax T (available from Novo Nordisk A/S) to obtain a mildly abraded look. The resulting L*a*b* values of the San Francisco denim after abrasion was: L*: 30.87; a*: -0.16; b*: -15.36

Dakota denim fabric (standard pure indigo denim fabric from Swift, France) was desized with Aquazym (available from Novo Nordisk A/S) and abraded with DeniMax T (available from Novo Nordisk A/S) to obtain a mildly abraded look. The resulting L*a*b* values of the Dakota denim after abrasion was: L*: 28.95; a*: 0.76; b*: -17.90.

**Abrasion enhancement using mCiP842-CBD**

The following was performed in a Wascator FOM 71 MP-LAB:
660 g denim (approximately 495 g of the above mildly abraded San Francisco denim, and approximately 165 g of the above mildly abraded Dakota denim),
16.5 litres of de-mineralised water,
12.38 g $KH_2PO_4$,
mCiP842-CBD (produced according to Example 1): 5.1 µg enzyme protein per g denim,
0.0825 g methyl syringate (Lancaster) corresponding to 0.60 µmole/g denim,
1.685 ml 30% $H_2O_2$ (Perhydrol, Merck) corresponding to 1 mM $H_2O_2$,
pH 6.0,
50°C,
30 minutes.

<u>The resulting L*a*b* values of the denim was:</u>
San Francisco denim: L*: 34.29; a*: -0.44; b*: -14.95.
Dakota denim: L*: 31.23; a*: 0.28; b*: -17.04.

<u>The change in L*a*b* was:</u>

San Francisco denim: ΔL*: 3.42; Δa*: -0.28; Δb*: 0.41.
Dakota denim: L*: 2.28; a*: -0.48; b*: 0.86.

From visual evaluation the abrasion enhancement process produced denim with a more worn (more abraded) look rather than a bleached look.

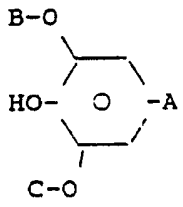
**CLAIMS**

1. A process for providing an abraded look with a reduced strength loss in dyed fabric comprising

(a) contacting, in an aqueous medium, a dyed fabric with a cellulase in a concentration corresponding to 0.01-250 µg of enzyme protein per g of fabric; and simultaneously or subsequently treating said fabric with a hybrid enzyme comprising a phenol oxidizing enzyme fused to amino acid sequence having a cellulose-binding domain together with a hydrogen peroxide source when the phenol oxidizing enzyme is a peroxidase, or

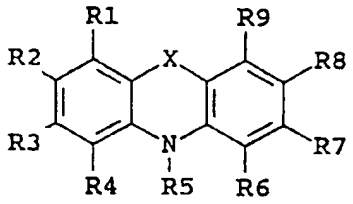
(b) contacting, in an aqueous medium, a dyed fabric with a hybrid enzyme comprising a phenol oxidizing enzyme fused to amino acid sequence having a cellulose-binding domain together with a hydrogen peroxide source when the phenol oxidizing enzyme is a peroxidase; and subsequently treating said fabric with a cellulase in a concentration corresponding to 0.01-250 µg of enzyme protein per g of fabric.

2. A process according to claim 1, wherein the aqueous medium additionally comprises an enhancing agent of formula I or formula II:
Formula I:



in which formula A is a group such as -D, -CH=CH-D, -CH=CH-CH=CH-D, -CH=N-D, -N=N-D, or -N=CH-D, in which D is selected from the group consisting of -CO-E, $-SO_2$-E, -N-XY, and $-N^+$-XYZ, in which E may be -H, -OH, -R, or -OR, and X and Y and Z may be identical or different and selected from -H and -R; R being a $C_1$-$C_{16}$ alkyl, preferably a $C_1$-$C_8$ alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and selected from $C_mH_{2m+1}$; $1 \leq m \leq 5$;

Formula II:




in which formula X represents (-O-) or (-S-), and the substituent groups $R^1$-$R^9$, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy, and esters and salts hereof, carbamoyl, sulfo, and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, $C_1$-$C_{14}$-alkyl, $C_1$-$C_5$-alkoxy, carbonyl-$C_1$-$C_5$-alkyl, aryl-$C_1$-$C_5$-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group $R^{10}$; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups $R^{10}$; and which $C_1$-$C_{14}$-alkyl, $C_1$-$C_5$-alkoxy, carbonyl-$C_1$-$C_5$-alkyl, and aryl-$C_1$-$C_5$-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups $R^{10}$;

which substituent group $R^{10}$ represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, $C_1$-$C_5$-alkyl, $C_1$-$C_5$-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, $C_1$-$C_5$-alkyl, $C_1$-$C_5$-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which $C_1$-$C_5$-alkyl, and $C_1$-$C_5$-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups $R^1$-$R^9$ may together form a group -B-, in which B represents any of the following the groups: $(-CHR^{10}-N=N-)$, $(-CH=CH-)_n$, $(-CH=N-)_n$ or $(-N=CR^{10}-NR^{11}-)$, in which groups n represents an integer of from 1 to 3, $R^{10}$ is a substituent group as defined above and $R^{11}$ is defined as $R^{10}$.

3. The process according to claim 1 or 2, wherein the fabric is dyed with a vat dye.

4. The process according to claim 3, wherein the vat dye is indigo or thioindigo.

5. The process according to claim 1 or 2, wherein the fabric is a cellulosic fabric or a mixture of cellulosic fibres or a mixture of cellulosic fibres and synthetic fibres.

6. The process according to claim 5, wherein the fabric is denim, preferably denim dyed with indigo or thio-indigo.

7. The process according to claim 1 or 2, wherein the cellulase is obtainable from Humicola, e.g., Humicola insolens, Fusarium, e.g., Fusarium oxysporum, Myceliophthora, e.g., Myceliophthora thermophila, or Cephalosporium sp.

8. The process according to claim 1 or 2, wherein the concentration of the cellulase corresponds to 0.1-50 µg of enzyme protein per g of fabric.

9. The process according to claim 1 or 2, wherein the phenol oxidizing enzyme is a peroxidase.

10. The process according to claim 9, wherein the peroxidase is horseradish peroxidase, soybean peroxidase or a peroxidase enzyme obtainable from Coprinus, e.g., C. cinereus or C. macrorhizus, or from Bacillus, e.g., B. pumilus, or Myxococcus, e.g., M. virescens.

11. The process according to claim 1 or 2, wherein the hydrogen peroxide source is hydrogen peroxide or a hydrogen peroxide precursor, e.g., perborate or percarbonate, or a hydrogen peroxide generating enzyme system, e.g., an oxidase and its substrate, or a peroxycarboxylic acid or a salt thereof.

12. The process according to claim 1 or 2, wherein the aqueous medium contains $H_2O_2$ or a precursor for $H_2O_2$ in a concentration corresponding to 0.001-25 mM $H_2O_2$.

13. The process according to claim 1 or 2, wherein the phenol oxidizing enzyme is a laccase or a laccase related

enzyme.

14. The process according to claim 13, wherein the laccase is obtainable from Trametes, e.g., Trametes villosa, Coprinus, e.g., Coprinus cinereus, or Myceliophthora, e.g., Myceliophthora thermophila.

15. The process according to claim 1 or 2, wherein the concentration of the hybrid enzyme corresponds to 0.01-250 µg of hybrid enzyme protein per g of fabric.

16. The process according to claim 2, wherein the enhancing agent belongs to the group consisting of acetosyringone, syringaldehyde, methylsyringate and syringic acid.

17. The process according to claim 2, wherein the enhancing agent belongs to the group consisting of 10-methylphenothiazine, phenothiazine-10-propionic acid, phenoxazine-10-propionic acid, phenoxazine-10-hydroxyethyl, phenothiazine-10-ethyl-4-carboxy, promazine hydrochloride and phenothiazine-10-ethylalcohol.

18. The process according to claim 2, wherein the enhancing agent in the aqueous medium is present in concentrations of from 0.05 to 500 µmole per g of fabric.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00176

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: D06M 16/00, C11D 3/386, D06L 3/02
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: D06M, C11D, D06L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAPLUS

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| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | WO 9407998 A1 (NOVO NORDISK A/S), 14 April 1994 (14.04.94) -- | 1-18 |
| A | WO 9218683 A1 (NOVO NORDISK A/S), 29 October 1992 (29.10.92) -- | 1-18 |
| A | WO 9412621 A1 (NOVO NORDISK), 9 June 1994 (09.06.94) -- | 1-18 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
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| 27 June 1997 | 08 -07- 1997 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Anna Sjölund<br>Telephone No. +46 8 782 25 00 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00176

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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Information on patent family members

03/06/97

International application No.
PCT/DK 97/00176

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